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Eco-enzyme Characterization and its Application as an Ammonia-Reducing Agent in Poultry Feces

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The level of air pollution due to livestock farming practices is increasingly worrying. The laying hen industry, as one of the industries with the largest population, is in the international spotlight. The production of ammonia gas (NH_3) from laying hen feces is suspected of playing an important role as a contributor to greenhouse gas (GHG) emissions. The existence of NH_3 is thought to be reduced by protease enzyme activity. Protease enzymes can be obtained from nature cheaply and simply and are easy to produce. One of them is by utilizing Eco-Enzyme (EE) products from fruit waste. This study aims to evaluate the character of EE from fruit waste and examine its effect in reducing NH_3 gas. This study uses basic material from laying hen feces as a source of NH_3 gas. The study is divided into 2 series of activities, namely (1) evaluating the character of EE from fruit waste and (2) evaluating the applied effect of EE on laying hen feces for a specific time. The study was conducted experimentally. A total of three ratios of fruit waste mixtures were used (1) 100% pineapple; (2) (pineapple + papaya) (ratio 50%:50%); (3) 100% papaya, as a source of EE and as many as four EE dilution ratios to be applied in the treatment, namely: P0 = 0% EE + 100% water (v/v)(control); P1 = 100% EE + 0% water (v/v); P2 = 90% EE + 10% water (v/v); P3 = 80% EE + 20% water (v/v). Reduction time is used 3 times, namely, M0 = 0th minute, M1 = 15th minute, and M2 = 30th minute. Data were processed statistically using a Completely Randomized Design with a Factorial pattern. Each treatment was repeated 3 times (4x3x3). Data were analyzed descriptively (enzyme character parameters) and statistically with ANOVA (NH_3 gas production, temperature and humidity kinetics). The results showed that the difference in the ratio of fruit waste mixture did not show a significant effect ($P>0.05$) on the pH value of EE, enzyme activity, and total LAB bacteria. Implementation of P1 (100% EE + 0% water) showed the highest effectiveness in reducing ammonia gas within 15 minutes after application to feces.
Keywords: Climate change, GHG emission, pollution, waste management, ammonia mitigation, bioremediation, fermentation.

INTRODUCTION

The results of the study presented by the United States Environmental Protection Agency (EPA), show that the most significant contributors of Greenhouse Gas (GHG) emissions are, electricity and heat production 25%, agriculture, forestry, and other land use 24%, industry 21%, transportation 14%, other energy 10% and buildings 6% of global GHG emissions in 2010. Indonesia's First Biennial Update Report (1st BUR) reported that total GHG emissions from all sectors increased by around 3.6% per year, one of which is the livestock sector, namely ammonia gas. Livestock is the largest producer of ammonia emissions into the atmosphere (Ikhwan *et al*., 2016). This issue is in line with the statement of Kementan (2014) that the large contribution of ammonia gas to the air is directly proportional to the increase in the world's poultry population; then in the same period, the population of laying hens increased by 368.19 million in 2021 (BPS, 2022). Yahya *et al*. (2017) stated that laying hens produces 0.06-0.15 kg/day of feces, while 100 grams of feces equals 0.54 ppm (Kementan, 2014). Ammonia, when mixed, reacts with acidic compounds in the air, increasing the number of aerosols that cause acid rain, which harms the environment, the productivity of laying hens, and human health, according to

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the statement (Yuwono, 2010)**.** Ammonia in the atmosphere reacts with nitrates and sulfates to form highly corrosive ammonium salts. The production of livestock industry waste can have negative impacts, but it can also have positive impacts if it can be utilized optimally. Livestock waste will provide value if the basic form of the raw material can be changed. This certainly requires process technology that requires basic knowledge (Said *et al*., 2018; Said *et al*., 2019; Said, 2019).

An innovative solution to mitigate ammonia gas pollution is the use of eco-enzymes (EE), which are produced through the fermentation of organic materials rich in microorganisms. EE are bioremediation agents that utilize enzymes (Tang and Tong, 2011) and active microorganisms produced during the fermentation process to degrade pollutants that are harmful to the environment (Win, 2011). Yusrizal *et al*. (2012) reported that using a combination of proteolytic, acid, and antibiotic-producing bacteria significantly reduced ammonia in feces during 24-hour incubation at room temperature.

This research uses pineapple and papaya skin waste. Previous research results have conveyed many efforts to reduce ammonia in water using EE from pineapple and papaya. The results of the study by Syahirah and Nazaitulshila (2019) showed that pineapple for aquaculture sludge treatment was reported to successfully reduce total ammonia by 50% in a 12-hour incubation period. Furthermore, Wikaningrum and Anggraina (2022) noted that EE based on papaya and spinach can reduce ammonia, nitrite, and nitrate contained in water.

Therefore, this study aims to characterize EE from fruit waste and determine the dilution ratio of EE that effectively reduces ammonia gas in laying hen feces. This study is expected to provide scientific information on the processing of fruit waste as an ammonia gas-reducing agent and provide a sustainable solution to the problem of greenhouse gas emissions from livestock.

MATERIALS AND METHODS

***Research materials*:** The materials used in making EE are 4.5 kg of pineapple, 4.5 kg of papaya that is unsuitable for consumption but has not rotted from the fruit market, 10 litres of water, and three litres of molasses obtained from the Takalar Sugar Factory. The materials used in ammonia reduction are the feces of the Hy-line brown laying hen strain obtained from the "Maros Biru" farm.

The tools used in making EE are a knife, a 10 kg capacity scale, a 25 L bucket, a sieve, and a basin, while the tools used in ammonia reduction are a plastic fibre cover measuring 40 cm x 20 x 33 cm, a glass feces container measuring 53 cm x 30 cm, sprayer (500 mL), and a Smart Sensor Ammonia Tester AR-8500 NH_3 Detector.

Research Methods

***a) Research design*:** The study was conducted experimentally. The study is divided into 2 series of activities, namely (1) evaluating the character of EE from fruit waste and (2) evaluating the applied effect of EE on laying hen feces for a certain time.

a.1. Characterization of EE product from fruit waste combinations .EE is produced by pineapple and papaya waste as a basic material. The basic formula applied to make EE is 10 liters of water + three kg of fruit waste + one kg of molasses (10:3:1). All ingredients are put into an airtight container. The fermentation process of fruit waste is carried out for 3 months. After the fermentation process, EE filtrate is produced. In this study, a total of three ratios of fruit waste mixtures were used (1) 100% pineapple (3 kg); (2) (pineapple + papaya) (1.5 kg + 1.5 kg) (ratio 50%:50%); (3) 100% papaya (3 kg), as a source of materials of EE. The stages of making EE can be seen in Figure 1.

a.2. Evaluating the application of EE on laying hen feces

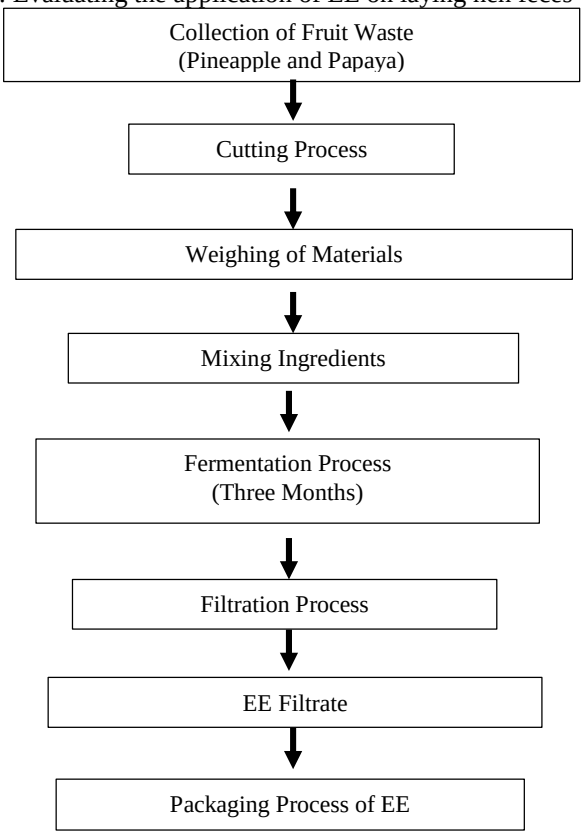

Figure 1. Flow chart of the production process of EE from fruit waste.

The research used a completely randomized design with a 4x3x3 factorial pattern. A total of 2 factors were applied in this study. The first factor is the application of four EE dilution ratios, namely: P0 = 0% EE + 100% water (v/v) (control); P1 = 100% EE + 0% water (v/v); P2 = 90% EE + 10% water (v/v); P3 = 80% EE + 20% water (v/v). The second factor is reduction time, namely, M0 = 0th minute, M1 = 15th

minute, and M2 = 30th minute. Each treatment was repeated 3 times.

***b) Implementation of EE on feces*:** Characterization of the best EE by looking at the highest protease enzyme activity of EE produced from different raw materials. The best EE will be applied to reduce ammonia gas in laying hen feces. A complete visualization of the implementation of EE in feces is presented in Figure 2.

c) Parameters and data analysis

***pH value*:** The pH value of EE is measured using a calibrated pH meter. Measuring the acidity level (pH) by inserting 100 mL of EE into a beaker and then inserting the tip of the pH meter sensor, the tool will display the pH value of the measured liquid.

***Protease enzyme activity*:** The activity of the protease enzyme was tested based on a 0.2 mL enzyme solution sample added with 1 mL of Tris-HCl buffer pH 7, then 1 mL of 20 mg/mL casein was added, then incubated at 37 °C for 30 minutes, then 1 mL of 0.1 M TCA was added. The control was made using the same procedure as the sample but without the direct incubation process; TCA was added to stop the enzyme activity. The supernatant (filtrate) of the sample and control was separated by centrifugation at 10,000 g for 5 minutes. The filtrate from the protease hydrolysis was taken as much as 1.5 mL, then 2.5 mL of 0.4 M Na_2CO_3 was added, 1 mL of 50% Folin was added, and then left for 30 minutes. After that, the absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer. The tyrosine standard and blank were made using the same procedure as the filtrate from the hydrolysis. The blank used was tris-HCl buffer pH 7. Enzyme activity was expressed in units (U), defined as the amount of enzyme required to release 1 µmol of tyrosine per minute at pH 7 and 37°C.

***Total of lactic acid bacteria (LAB)*:** Calculation of total Lactic Acid Bacteria using the SPC method (*Standard Plate Count*): as much as 1 mL of the sample was diluted into 9 mL of 0.85% physiological NaCl from 10^{-1} - 10^{-4}, then the last three dilution series (10^{-2}, 10^{-3}, and 10^{-4}) were taken as much as 1 mL and inoculated by pour plate on MRS Agar medium supplemented with 1% $CaCO_3$ in a petri dish, then incubated for 48 hours in an incubator at 37°C. The growth of lactic acid bacteria on MRS Agar medium supplemented with $CaCO_3$ was indicated by the presence of a clear zone around the bacterial colony. The grown lactic acid bacteria were counted, and the total number was calculated using the Total Plate Count (TPC) method (Amaliah *et al*., 2018).

***Ammonia gas reduction*:** Ammonia gas measurements were taken 20 minutes after a lid covered the stool. Observation and data collection of temperature were carried out by observing the temperature data on the Smart Sensor Ammonia Tester Detector (AR-8500 NH_3) at minute 0th, minute 15th, and minute 30th after spraying of EE.

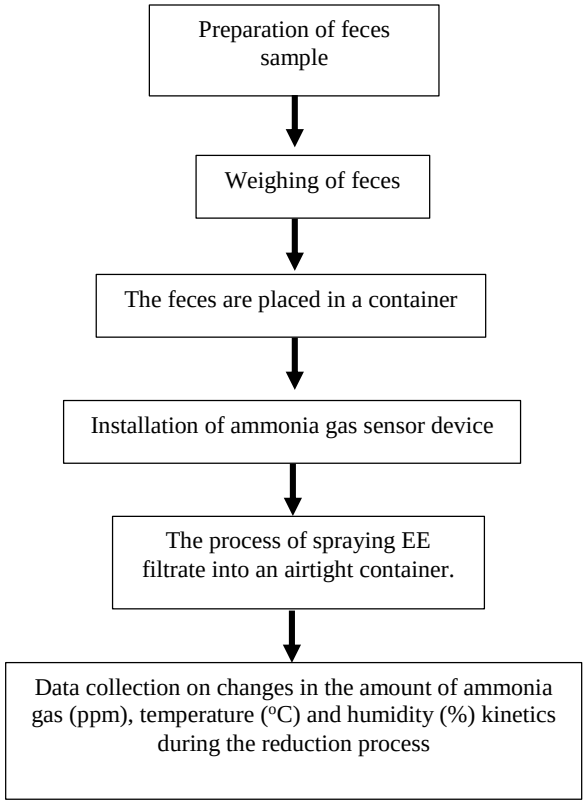

Figure 2. Flow chart of the implementation of EE in the laying hen feces.

***Temperature kinetics (°C)*:** Temperature measurement is done 20 minutes after feces are covered using a lid. Observation and data collection of temperature were carried out by observing the temperature data on the Smart Sensor Ammonia Tester Detector (AR-8500 NH_3) at minute 0, minute 15, and minute 30 after spraying of EE.

***Humidity kinetics (%)*:** Humidity measurements were performed 20 minutes after the feces were covered using a lid. Observation and taking of humidity were carried out by observing humidity data on the hygrometer at minute 0th, minute 15th, and minute 30th after praying EE.

***d) Statistical analysis*:** Observation data of analysis of variance based on Completely Randomized Design (CRD) factorial pattern (4x3x3). The results of the variance analysis that show a significant effect will be continued with the Duncan test using IBM SPSS Statistics 25.0 software. If the treatment shows a real impact, it is continued with the Duncan test (Gaspersz, 1991).

RESULTS

***pH value*:** The results of the pH level test produced from three different EE fermented for 3 months are presented in Figure 3.

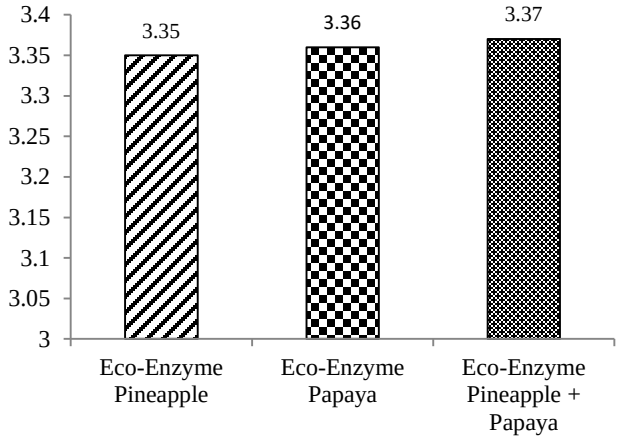

Figure 3. Differences in the pH value of EE obtained from the fermentation of fruit waste.

Figure 3 shows the pH of EE of pineapple 3.35 and papaya 3.36 shows no significant difference in pH, and the combination of Pineapple + Papaya 3.37, indicating that the combination of the two materials gives a slightly increased effect on pH, it can be seen that the pH of EE produced from different basic materials is in the range of <4.

***Protease enzyme activity*:** The results of the protease enzyme activity test on EE based on pineapple, papaya, and pineapple + papaya can be seen in Figure 4.

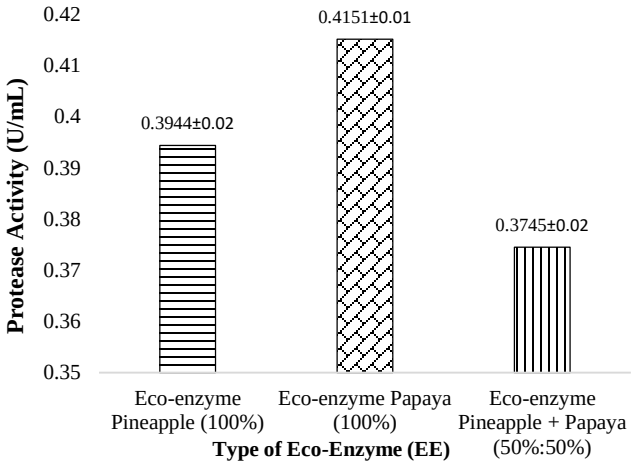

Figure 4. Comparison of EE protease enzyme activity (U/mL) on several combinations of fruit waste materials.

Figure 4 shows that the activity enzyme protease in EEs produced from different raw materials measures din units per milliliter (U/mL). Pineapple EE 0.3944 U/mL, papaya EE 0.4151 U/mL, Pineapple + Papaya EE 0.3745 U/mL.

***Total of lactic acid bacteria (LAB)*:** The results of the total Lactic Acid Bacteria (LAB) test on EE based on pineapple, papaya, and pineapple + papaya can be seen in Figure 5.

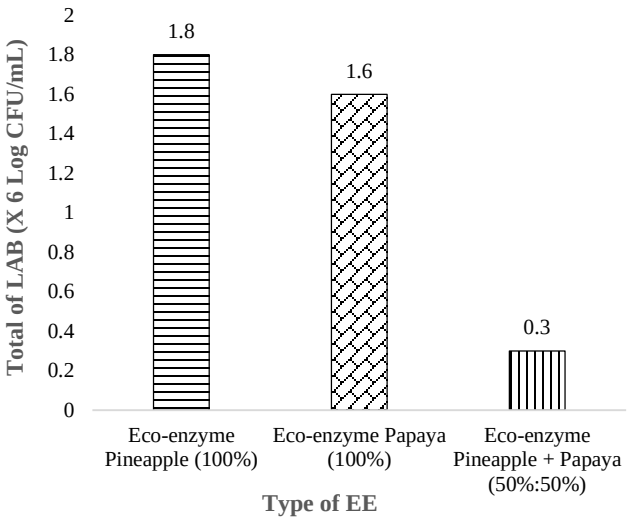

Figure 5. Comparison of total of LAB (6 Log CFU/mL) on several combinations of fruit waste materials.

Based on Figure 5 shows the total bacteria presented (log CFU/mL) for three different EE solutions: pineapple EE 1.8 x 6 Log CFU/mL, Papaya EE 1.6 x 6 Log CFU/mL, Pineapple + Papaya EE 0.3 log CFU/mL.

***Dynamics of ammonia gas*:** The results of applying papaya-based EE in reducing ammonia gas in laying hen feces are presented in Figure 6.

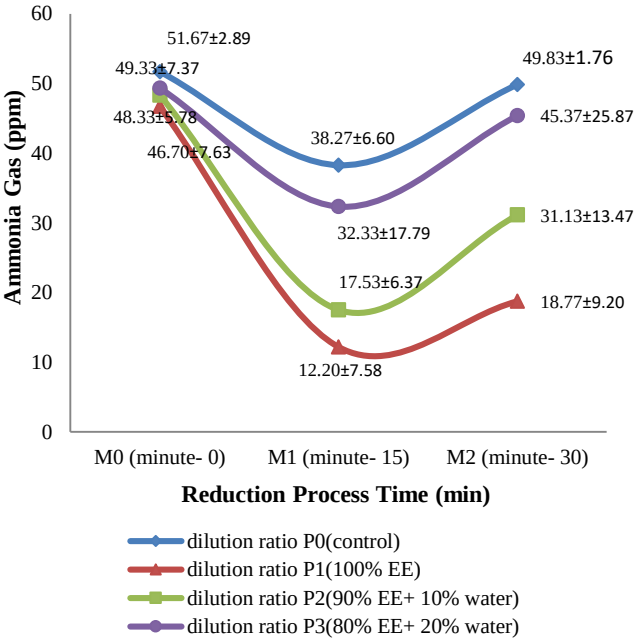

Figure 6. Dynamics of changes in ammonia gas production (ppm) from laying hen feces during the reduction process using EEs from fruit waste.

Based on Figure 6, shows that all treatments, including the control, showed degradation of ammonia gas in the feces. Negative control shows that without EE administration, the ammonia gas levels in the feces still decreased but not as significantly as those applied to EE. The administration of 100% EE could degrade ammonia gas significantly in the 15th minute.

***Temperature kinetics (°C)*:** The dynamics of temperature changes during the fermentation process in laying hen feces after the complete administration of EE are presented in Figure 7.

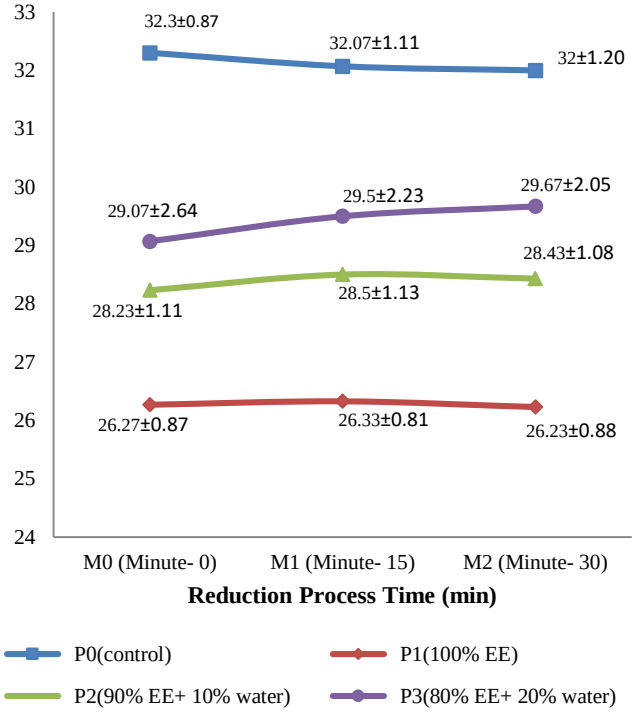

Figure 7. Dynamics of changes in temperature kinetics (ºC) from laying hen feces during the reduction process using EE from fruit waste.

Based on Figure 7, the application of EE on feces showed different temperature changes along with the concentration level of EE used.

***Humidity kinetics (%)*:** Changes in humidity during the fermentation process in laying hen feces after administration of EE are seen in Figure 8.

Based on the graph in Figure 8 shows that all treatments have varying graph patterns. A unique increase in humidity occurs in the first 15 minutes (from minute 0th to minute 15th), indicating that the EE is reacting. EE 100% and 90% produce a constant graph in the first 15 minutes, while EE 80% and Control experience an increase in the 15th minute.

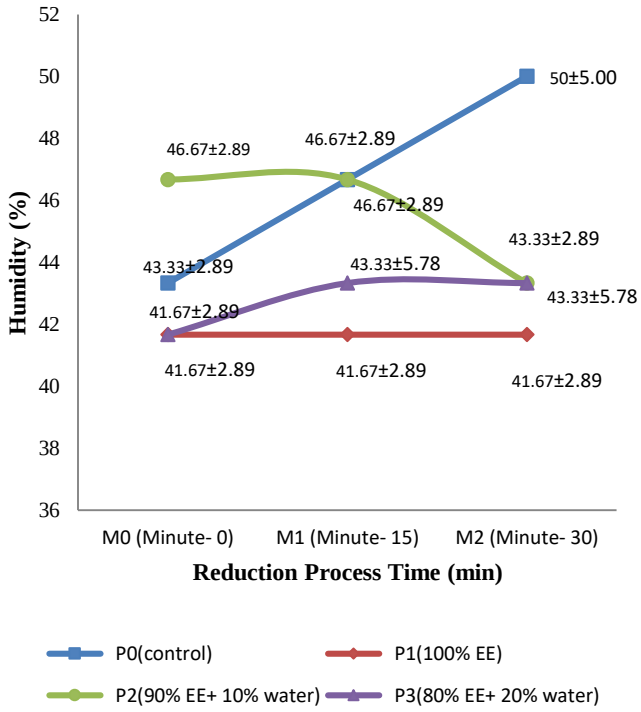

Figure 8. Dynamics of changes in humidity kinetics (%) from laying hen feces during the reduction process using EE from fruit waste.

DISCUSSION

The strategy for processing fruit waste into EE products can be done in several stages, such as 1) collecting fruit waste, Which can be collected from markets, restaurants, or households; 2) cleaning and crushing. Fruit waste is cleaned and crushed to obtain nutrient-rich extracts; 3) adding microorganisms. Microorganisms such as yeast or bacteria are added to the extract to facilitate fermentation; 4) Fermentation process. Fermentation takes several days to produce eco-enzymes rich in nutrients; 5) drying and packaging process. The resulting eco-enzymes can be dried and packaged in a form ready to be applied.

The data in Figure 3 indicates that the EE produced is at a neutral pH. The results of characterization observations on pH show a pH below 4, meeting good standards in making EE (Putra and Suyasa, 2022). This shows that the eco enzyme produced is still by good standards. The low pH of the EE produced is caused by the high content of organic acids such as citric and acetic acids (Etienne *et al*., 2013). Rasit *et al*. (2019) said that the greater the organic acid content in EE, the lower the pH of EE; thus, it can be concluded that the low pH of EE is caused by the high organic acid content, namely acid. Citric and acetic acid are EE, so the raw materials provide good EE results.

The analysis results on Figure 4 showed that Papaya EE has the highest protease activity among the three types of EE produced;. However, pineapple EE contains less protease enzyme than papaya EE, it still shows protease enzyme activity significant. This is because the papain enzyme found in papaya fruit is more resistant to temperature compared to the bromelain enzyme in pineapple fruit; this is to previous research that the papain enzyme is more resistant to high temperatures compared to the bromelain enzyme (Winarno, 1995).

The data in Figure 5 shows that the three EEs produced have various bacteria and indicate the activity of acid bacteria involved during the fermentation process. Pineapple EE produces the highest number of bacteria compared to a single papaya and a combination of pineapple and papaya. This shows that a single-based EE contains more lactic acid bacteria than a combination (Samriti and Arya, 2019). Acetic acid was found in EE, supported by research (Larasati *et al*., 2020). Acetic acid comes from bacterial metabolism found in vegetable and fruit waste; this process is anaerobic metabolism, namely bacterial fermentation, to obtain energy from sugar, which obtains by-products of acetic acid and alcohol. Then Pelczar and Chan (2005) said that during the fermentation process, the condition of the LAB activity medium is influenced by temperature and pH. In terms of safety, using EE can be very safe. 1) Toxicity. EE products produced from fruit waste do not have toxicity that harms humans and the environment; 2) Allergies. EE products very rarely cause allergies in humans; 3) Contamination. EE products are not easily contaminated with other bacteria or fungi due to the role of Lactate Acid Bacteria (LAB); 4) Dosage. Using EE enzymes in the right dose is crucial to avoid side effects. Several testing steps can be carried out as preventive measures: 1) Toxicity testing. Toxicity testing is carried out to determine whether eco-enzymes have harmful effects on humans and the environment; 2) Allergy testing: Allergy testing is carried out to determine whether EE can cause allergies in some people; 3) Contamination testing: Contamination testing is carried out to determine whether EE is easily contaminated with other bacteria or fungi.

Figure 6 indicates that the 100% EE treatment is more effective than the negative control in reducing ammonia gas levels in the first 15 minutes after spraying, while administration of 90% EE is more effective than 80% EE and administration of 80% EE is more effective than the negative control in reducing ammonia gas. This is in line with research by Nazim (2013). Higher concentration levels and longer exposure times resulted in more significant reductions in ammonia concentrations in wastewater than in (Syahirah *et al*., 2019). In his research, it was reported that the processing of aquaculture sludge successfully reduced total ammonia by 50% with a longer incubation time. Rasit *et al*. (2019) said that the degradation reaction of ammonia gas in feces occurs due to the content of protease enzymes as proteins in EE as catalysts that catalyze complex organic materials into simpler ones and help stabilize organic materials into substances that are more easily dissolved and decomposed.

Processing fruit waste into EE is an effort to process fruit waste, which is waste, into something useful so that it can avoid environmental pollution. One of the contributors to GHG emissions is ammonia. Ammonia is an air pollutant in livestock, and livestock is the largest producer of ammonia emissions into the atmosphere (Ikhwan *et al*., 2016). Ammonia, when mixed, reacts with acidic compounds in the air, increasing the amount of aerosols that cause acid rain, which is very dangerous for the environment, the productivity of laying hens, and human health. According to the statement Yuwono (2010), ammonia in the atmosphere reacts with nitrates and sulfates to form highly corrosive ammonium salts. In this study, EE made from pineapple and papaya waste acts as an ammonia gas-reducing agent in livestock, especially in laying hens. The EE produced in this study can reduce ammonia gas in the feces of laying hens. Previous research stated that using EEs for aquaculture sludge treatment reduced total ammonia by 50% with a longer incubation time (Syahirah *et al*., 2019). This occurs due to the protease enzyme content contained in EE,

Figure 7 shows that each concentration level now gives a relatively insignificant change trend, along with the walk time from minute 0 to minute 30. The control showed a small but constant decrease until the end of the measurement. EE 100% started at a temperature of 26.27°C, then increased at minute 15 and decreased again at minute 30 after spraying. EE 90% shows a similar pattern with an initial temperature of 28.23°C, which increases at the 15th minute and decreases again at the 30th minute. EE 80% shows a relatively small but constant increase in temperature from the 0th minute to the 30th minute. The graph above can assume that the diluted EE affects the temperature but not too much significantly during the measurement period from minute 0 to minute 15; each treatment experienced an increase in temperature except for the negative control EE; this indicates that spraying EE on the feces reacts to reduce ammonia. Protease enzymes work optimally with an increase in optimal temperature; however, temperatures that are too high (far from the optimum temperature of an enzyme) will cause the enzyme to denature (Poedjiadi, 1994).

The application of EE to the feces of laying hens to reduce ammonia gas with a concentration of 80% and 90% EE is not as effective as 100% EE in reducing ammonia levels in the feces of laying hens in the first 15 minutes and will rise again in the 30th minute. The temperature kinetics of the application of laying hen feces showed a stable temperature in each treatment, namely P0:32°C, P1:26°C, P2:28°C, P3:29°C and, humidity kinetics show a sequential graph, namely P0, P3, P2, P1 d so that it can be concluded that the administration of EE affects feces, this is in line with In addition to the protease enzyme content in EE, air, and humidity levels also affect the

process of reducing ammonia gas in feces, this is in line with (Bleizgys and Naujokiene, 2023), ammonia emissions are strongly influenced by air temperature and relative humidity, with emission levels tending to increase with increasing temperature and then decrease with increasing humidity. Moreover, Mokoena *et al*. (2006) revealed that increased urease activity is very sensitive to feces temperature and is the cause of high ammonia concentrations in feces, where feces temperature is very dependent on air temperature.

Figure 8 indicates that EE with a high concentration can maintain humidity more effectively. EE with a concentration of 80% and Control experience an increase caused by the dilution of EE affecting efficiency in maintaining humidity; therefore, adding water with a higher concentration level into the EE, reduces the ability of EE in retaining moisture. Low humidity results in reduced solubility of nutrients in the substrate. High humidity that exceeds the optimum limit can reduce the effectiveness of enzyme work, which causes the degradation process to be slower (Dias *et al*., 2007). The optimum humidity for protease enzymes is around 65% (Haque *et al*., 2016). Based on the research results that have been carried out in two stages, the first is the manufacture of EE from different raw materials. The second is its application to the feces of Hy-line Brown laying hens to reduce ammonia gas; in this study, several technicalities cannot be avoided, namely rain, strong winds, ammonia levels in feces, and limited equipment, so that the research cannot run optimally. EE use on feces is expected not only for laying hens but also to expand to other poultry and ruminant sectors. In addition, household waste that is widely found around us can be utilized by making EEs from fruit and vegetable materials. The use of EE on a large scale in the poultry industry can be done manually or using spray technology that can be programmed automatically. This spray can be installed on every corner or pole of the cage and programmed at any desired time. Applying EE is very easy because the raw material is only fruit waste so it is easy to obtain and cheap. The manufacturing method is also quite simple and fast, making it easy for the community to adopt. In addition, Eco-Enzyme can be mass-produced to make it very visible and implementable.

Some of the advantages of utilizing fruit waste as raw material in eco-enzyme production include: 1) the availability of this waste is quite abundant. Fruit waste is available in large quantities and can be obtained easily and cheaply; 2) Low cost. Fruit waste can be obtained cheaply or without payment; 3) high nutritional content. Fruit waste contains high nutrients, such as sugar, amino acids, and minerals.

The production process of EE from fruit waste is economically very visible. Some basic data in its economic calculations, namely: 1) Raw material costs: IDR 18,000/kg (fruit waste, molasses); 2) Process costs: IDR 80,000/day (labour, electricity, equipment); 3) Other costs: IDR 15,000/kg (packaging, distribution), resulting in a total cost of: IDR 113,000/kg. Analysis of income that can be obtained from EE products includes: 1) Selling price: IDR 50,000/litre; 2) Production volume: 100 litres/day; 3) Income: IDR 5,000,000/day (100 litres x IDR 50,000/litre). Based on the cost data and income analysis, the profit analysis is then calculated, namely: 1) profit: IDR 5,000,000/day (income) - IDR 113,000/kg x 100 kg (total cost) = IDR 3,887,000/day; 2) profit margin: 77.74% (IDR 3,887,000/IDR 5,000,000); 3) BEP: IDR 113,000/kg x 100 kg/IDR 50,000/litre = 226 litres; 4) BEP time: 2.26 days (226 litres/100 litres/day).

***Conclusion*:** The results showed that the concentration (P1: 100% EE + 0% water) produced better characteristics than other treatments (P0: Control, P2: 90% EE + 10% water) and P3: 80% EE + 20% water) in terms of ammonia gas production, kinetic temperature, and kinetic humidity. The 15-minute time after the spraying process showed a maximum decrease in ammonia gas.

***CRediT author statement*:** Conceptualization: A.H. Kadir; M.I. Said; validation: A.H. Kadir; M.I. Said; A. Asriany. writing: A.H. Kadir, M.I. Said; review and editing: A. Asriany; visualization: A.H. Kadir; M.I. Said; supervision: A.H. Kadir; M.I. Said; A. Asriany; reviewed and finalized the draft

Conflict of interest: All authors declare no conflict of interest.

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***Ethics statement*:** The ethical issues were addressed as per designed procedures.

Availability of data and materials: All data is published in this article.

Consent for publication: All authors submit their consent to publish this research article in JGIAS.

***Informed consent*:** N/A

SDGs addressed: No Poverty, Zero Hunger.

***Policy referred*:** National Action Plan for Greenhouse Gas Emission Reduction (RAN-GRK), National Livestock Waste Management Policy, Circular Economy Policy and Waste-to-Resource Initiatives.

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